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Short communication

Two new diterpenoids from *Leonurus japonicus*

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ABSTRACT

Two new labdane-type diterpenoids, 5-ethoxy-3-(2-((*R*)-4-hydroxy-2,5,5,8a-tetramethyl-3-oxo-3,5,6,7,8,8a-hexahydronaphthalen-1-yl)ethyl)furan-2(5H)-one (**1**), and 5-hydroxy-4-(2-((1*R*,3*S*,7a*S*)-1-hydroxy-4,4,7a-trimethyl-3-propionyloctahydroisobenzofuran-1-yl)ethyl)furan-2(5H)-one (**2**), were isolated from the EtOH extract of the herb of *Leonurus japonicus* Houtt., Lamiaceae. Their chemical structures were determined by analysis of 1D, 2D NMR, HRESIMS, and by comparison with known analogs.

Introduction

Leonurus japonicus Houtt., Lamiaceae, is an annual or biennial herbaceous plant widely distributed and cultivated in China. The dried herb is used in TCM for the treatment of various diseases, such as menstrual disturbances, dysmenorrhea, and amenorrhea (Liu et al., 2014). Recently, phytochemical studies on this plant have been reported (Romero-González et al., 2006; Ye et al., 2014; Fuchino et al., 2013; Chang et al., 2010; Moon, 2010; Xiong et al., 2013a,b; Zhang et al., 2013; Peng et al., 2013). Our previous investigation on two plants of the family of Lamiaceae resulted in the isolation of a number of new labdane diterpenes (Wu et al., 2011, 2013). In our research on psychoactive natural products from *L. japonicus*, two diterpenoids were isolated and identified. Their chemical structures for **1** and **2** were shown. Herein, we report the isolation, structural elucidation of these compounds.

Materials and methods

NMR spectra were recorded on a Bruker Avance III spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. HRESIMS were measured with Waters UPLC-LCT Premier XE and was controlled by MassLynx 4.1 software. Optical rotations were acquired with a WZZ-2ss automatic polarimeter (Shanghai Shenguang High Strength Bolts Co., Ltd., China). Column chromatography was performed with silica gel (200–300 mesh, Yantai Institute of Chemical Technology, Yantai, China), Sephadex LH-20 (GE Healthcare). HPLC separation was performed on an instrument (LC-3000, Beijing Chuangxintongheng Science & Technology Co., Ltd., Beijing, China) consisting of two pumps and a UV/vis detector with an YMC-ODS-A (150 mm × 20 mm) preparative column packed with C_{18} (5 μm).

The herb of *L. japonicus* Houtt., Lamiaceae, was purchased from Zhangye, Gansu Province of China in March, 2014. It was identified by one of the authors (Dr. H. Wu). A voucher specimen (Code: hkwu-aynu-20140301) was deposited at the Pharmaceutical Research Lab, Anyang Normal University.

The dried and powdered herb of *L. japonicus* (85 kg) was extracted with 95% EtOH (3× 400 l) by percolation at room temperature. The solvent was evaporated under reduced pressure at 45 °C to yield 7.2 kg viscous syrup, which was dissolved in water and extracted with EtOAc to get 3 kg extract. The EtOAc fraction was suspended in 30% EtOH and allowed to pass through a column (30 cm × 100 cm) packed with AB-8 macroporous resin (10 kg), then eluted with 50%, 70%, 90%, and 95% EtOH to obtain fractions A–D. Fraction C (693 g) was separated by silica gel CC over petroleum ether–EtOAc (10:1, 4:1, 2:1, 1:1) to yield subfractions (C1–C4). The further separation of fraction C2 with Sephadex LH-20 (MeOH), and then separated by reversed-phase preparative HPLC (60% acetonitrile in water, at a flow rate of 3 ml per min) to yield pure **1** (9 mg) and **2** (38 mg). In addition, a known compound (+)-sesamin, a lignan, was precipitated from the concentration of the eluate of the Sephadex LH-20 column.

5-Ethoxy-3-(2-((R)-4-hydroxy-2,5,5,8a-tetramethyl-3-oxo-3,5,6,7,8,8a-hexahydro-naphthalen-1-yl)ethyl)furan-2(5H)-one (**1**): Light yellow oil, $[\alpha]_D^{20} = -0.33$ ($c = 0.1$, MeOH); ^{1}H and ^{13}C NMR, see Table 1, HR-ESIMS m/z 375.2153 $[M+H]^+$ (calcd. 3375.2171).

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Table 1
^{1}H NMR and ^{13}C NMR data of **1** and **2** (400 and 100 MHz; acetone-d_6, δ ppm, J in Hz).

Position	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
1	29.4	1.50 (m), 2.05 (m)	30.7	1.40 (m), 1.60 (m)
2	17.1	1.69 (m), 1.87 (m)	18.9	1.58 (m), 1.68 (m)
3	37.2	1.90 (m)	41.1	1.18 (m), 1.45 (m)
4	35.7		32.1	
5	140.6		54.0	2.54 (d, 11.6)
6	143.1		83.5	4.40 (d, 11.6)
7	181.5		211.5	
8	127.9		31.6	2.77 (q, 7.2)
9	164.3		109.8	
10	43.9		48.9	
11	28.4	2.51 (m), 2.64 (m)	30.4	1.74 (m); 2.15 (m)
12	24.3	2.46 (m)	21.5	2.95 (m), 2.83 (m)
13	137.4		167.1	
14	142.4	6.88 (br s)	112.9	5.91 (d, 2.0)
15	101.6	5.84 (br s)	170.8	
16	170.9		97.3	6.19 (s)
17	11.5	1.98 (s)	6.9	1.06 (t, 7.2)
18	28.0	1.38 (s)	33.1	0.79 (s)
19	27.7	1.38 (s)	22.2	1.07 (s)
20	27.8	1.38 (s)	16.2	1.09 (s)
—OH		6.96 (s)		
O—$\underline{CH_2}$—CH_3	65.2	3.84 (m)		
O—CH_2—$\underline{CH_3}$	14.5	1.29 (m)		

5-Hydroxy-4-(2-((1R,3S,3aS,7aS)-1-hydroxy-4,4,7a-trimethyl-3-propionyloctahydroiso-benzo-furan-1-yl)ethyl)furan-2(5H)-one (**2**): Colorless oil, $[\alpha]_D^{20}$ = +55.4 (c = 0.2, MeOH); ^{1}H and ^{13}C NMR, see Table 1, HR-ESIMS m/z 367.2113 $[M+H]^+$ (calcd. 367.2121), 389.1928 $[M+Na]^+$ (calcd. 389.1940).

Results and discussion

A 95% ethanol extract of *Leonorus japonicus* Houtt., Lamiaceae, was suspended in water and extracted with EtOAc. The EtOAc fraction was subject to macroporious resin (AB-8) column chromatography (CC), and then to silica gel CC, Sephadex LH-20 CC, and HPLC, affording two new compounds.

Compound **1** was obtained as a yellowish oil. Its molecular formula was determined as $C_{22}H_{30}O_5$ on the basis of HRESIMS ($[M+H]^+$ 375.2153), indicating eight degrees of unsaturation. The ^{1}H and ^{13}C NMR (Table 1) showed the presence of one ethoxy group, and twenty additional carbon resonances, attributed to four tertiary methyl groups, five methylenes, two methines with one sp^2 carbon (δ 6.88/142.4) and one acetalic (δ 5.84/101.6), and nine quaternary carbons (with seven sp^2 carbons, an α, β-unsaturated ketone as revealed by the low field signals at δ_C 164.3, 181.5, and a lactone carbonyl at δ_C 170.9). These data suggested that **1** was a labdane-type diterpenoidal compound. These data were comparable to those of leojaponin (Romero-González et al., 2006), with similar resonances for the A and B rings. The ^{13}C NMR resonances at δ_C 170.9, 142.4, 137.4, 101.6, 65.2, and 14.5 and the ^{1}H NMR resonances at δ_H 6.88 (1H, s), 5.84 (1H, s), 3.84 (2H), and 1.29 (3H) indicated that **1** possessed a 3-substituted 5-ethoxyfuran-2 (5*H*)-one moiety at C-12 rather than the furan ring of leojaponin. The signal of a hydroxyl group was easily deduced, as there was no HMQC correlation between the hydroxyl proton (δ_H 6.96 *s*) and any other carbon, and the HMBC spectrum showed the correlations of the hydroxyl proton with three sp^2 carbons (δ_C, 143.1, 140.6, and 181.5), and also the HMBC correlations (Fig. 1) of H_3-17 with C-7, C-8, and C-9 allowed us to determine the only possible partial structure was C=C(OH)—C(C=O)—C(CH$_3$)=C, which was identical to those of leojaponin. A comparison of the NMR spectroscopic data of the C-11 to C-16 region of **1** to those reported for the structural analog sibiricinone C (Boalino et al., 2004) facilitated assembly of its C-11 to C-16 component. However the only C-15 relative configuration could not be assigned. Thus, the molecular structure of **1** was determined as 5-ethoxy-3-(2-((*R*)-4-hydroxy-2,5,5,8a-tetramethyl-3-oxo-3,5,6,7,8,8a-hexahydronaphthalen-1-yl)ethyl)furan-2(5*H*)-one.

Compound **2** was obtained as a colorless oil, and the molecular formula was determined as $C_{20}H_{30}O_6$ on the basis of HRESIMS ($[M+H]^+$ 367.2113; calc. 367.2121), indicating eight degrees of unsaturation. The ^{1}H and ^{13}C NMR (Table 1) showed the presence of twenty carbon resonances, attributed to one secondary methyl group (δ 1.06/6.9), three tertiary methyl groups (δ 0.79/33.1, 1.07/22.2, 1.09/16.2), six methylenes, four methines with one sp^2 carbon (δ 5.91/112.9) and two oxygenated (δ 6.19/97.3, 4.40/83.5), and six quaternary carbons. These data were comparable to those of *seco*-labdane (Rustaiyan et al., 1995), with similar resonances for the A and B rings. The ^{13}C NMR resonances at δ_C 170.8, 167.1, 112.9, and 97.3 and the ^{1}H NMR resonances at δ_H 5.91 (1H, d, J = 2.0 Hz), 6.19 (1H, s) indicated that **2** possessed a 3-substituted 5-hydroxyfuran-2 (5*H*)-one moiety, similar to sibiricinone A (Boalino et al., 2004) at C-12 rather than the furan ring of *seco*-labdane. The molecular structure of **2** was confirmed by the HMBC correlations

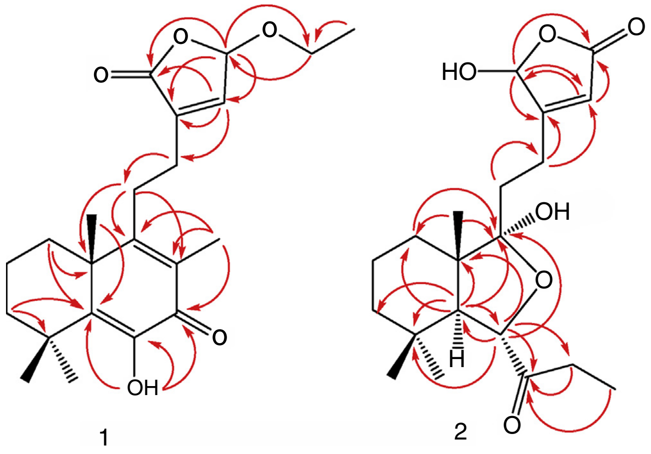

Fig. 1. Selected HMBC correlations of **1** and **2**.

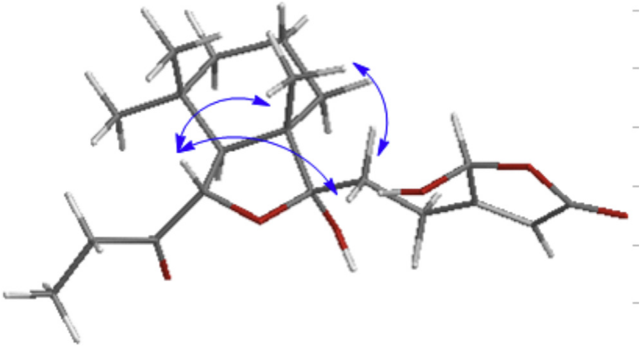

Fig. 2. Key NOESY correlations of **2**.

between H_3-20 and C-1/C-5/C-9; H_3-18 and C-4/C-5/C-19; H_3-17 and C-7/C-8; H-5 and C-1/C-3/C-4/C-6/C-7/C-9/C-10; H-6 and C-4/C-5/C-7/C-8/C-9/C-10; H-11 and C-9/C-12/C-13; H-12 and C-11/C-13/C-14/C-16; H-14 and C-12/C-13/C-15/C-16; H-16 and C-12/C-13/C-14/C-15 (Fig. 1). The relative configurations at C-5, 6, 9 as assigned via NOE association were the same as the reported those of *seco*-labdane (Fig. 2). Therefore, the structure of **2** was defined as 5-hydroxy-4-(2-((1*R*,3*S*,3a*S*,7a*S*)-1-hydroxy-4,4,7a-trimethyl-3-propionyloctahydroisobenzofuran-1-yl)ethyl)furan-2(5*H*)-one.

The structure of compound **1**, bearing an ethoxy group at C-15, must be an artifact of isolation, produced during the extraction procedure with EtOH from the naturally occurring hemiacetal. Yan-Ping Shi et al. have reported several labdane-type diterpenoids with ethoxy and methoxy groups from *L. heterophyllus*, and indeed, they could not find the same compounds when the dry plant was extracted by CH_2Cl_2 and monitored by HPLC analysis (Gong et al., 2012). Therefore, it can be predicted that if protic solvents (such as ROH) were used, corresponding products with alkoxy groups might be generated. The formation of compound **2** might be easily explained via a retro aldol reaction starting from a similar derivative of **2** (Rustaiyan et al., 1995).In conclusion, the EtOH extract of the herb of *L. japonicus* was purified by multiple chromatographic methods. Two diterpeniods were isolated and their chemical structures were elucidated, and one of them has an α, β-unsaturated lactone moiety, whose structure is similar to leojaponin (with a cross-conjugated α, β-unsaturated ketone moiety) except for the lack of a furanic ring. *O*-ethylation is unusual in plants biosynthesis; therefore, compound **1** is an artifact from the extraction process with ethanol. To the best of our knowledge, α, β-unsaturated carbonyl groups function as potent Michael receptors, which can react with nucleophilic reagents, thus this compound might have potent affinity to a series of receptors or enzymes.

Conflicts of interests

All authors declare that there are no conflicts of interests and they affirm that this paper consists of original and unpublished work.

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